3.8.2015 Interlab measurement

MONDAY, 8/3

Markins in tubes: D1 L A 3.8.2015 (Older restrictions J23101+I13504 22.7), D3 L A 3.8.7.2015 (J23117+I13504 Older restrictions 22.7), D1 L B 3.8.2015 and D3 L B 3.8.2015 (Newer restrictions 30.7)

where the "L" means "Ligated"

Did the ligation for the restricted parts of J23101,I13504 and J23117 creating D1 (J23101+I13504) and D3 (J23117+I13504) with both old restrictions made on 22.7 and new ones on 28.7. Kept all enzymes and buffer.

Made the following restriction mixtures:

Table1

DNA sample	Volume of insertion (ul)
AHD1 R	5,084477143
AHD3 R	5,084477143
AHD4 R	7,136864
AHD5 R	5,0

Added the required amounts of insertion, 2ul of 10x T4 DNA ligase buffer and 0,2 ul T4 DNA ligase to each tube and balanced total volume to be 20 ul with nuclease-free water. After the addition of ligase, there was 20 min pause when the tubes were in ice before starting incubating 20 mins at 22C. The ligase was inactivated at 65C for 10 mins and put on ice.

Made the transformation for four ligations: D1 A, D1 B, D3 A and D3 B.
Added 50 ul of ice cold compenents cells into pre-chilled 2ml tube and 2 ul of resuspended DNA to the same tube, and mixed with pipette gently. Liquid was transparent and there was some moisture on the tube walls. The cells were incubated on ice for 30 mins. The tubes were put in a water bath (42C) for 60s. After the heat shock the cells were on ice incubation for 5 minutes. Incubated the cells at 37C for 1 hr with shaking (230 RPM). At this point there seemed to be growth in tubes, because the liquid turned to be bleary.

4.8.2015 Interlab measurement

TUESDAY, 8/4

Checked yesterday's plates D1A, D1B (J23101+I13504), D3A and D3B (J23117+I13504) under the UV-light. D1A had some GFP expressing colonies which were gathered for overnight liquid culture and plate screening. Other plates contained only original colonies so they were put back in to 37C incubation at 11.00 and taken out 16.00. GFP colonies detected after the afternoon incubation from the plate D1B.

The wrong amount of XbaI was added earlier so it needs to be checked with the gel electrophoresis. Made an 1,25% agarose gel by adding 0,625 agarose into 50ml 1xTAEE. Did the gel electrophoresis for restricted part AHD4 (30.7) with the 120V potential for 25 mins. The result can be seen Fig 1 where the well 2 represents the sample:

Geldoc_2015-08-04_17hr_11min_interlab_ahd4.jpg

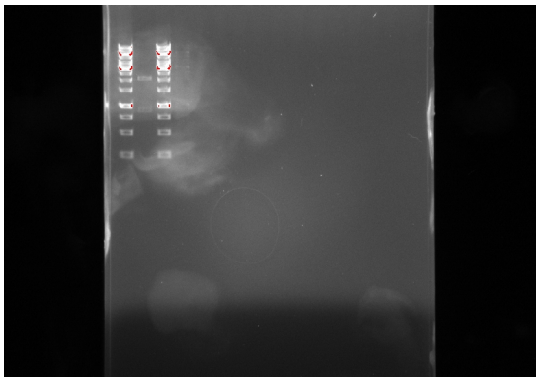

Fig 1. Gel electrophoresis for restricted AHD4

The size of AHD4 seems to be right (875 bp) so the restriction procedures can be assumed done properly.

5.8.2015 Interlab measurement

WEDNESDAY, 8/5

Checked yesterdays fresh culture plates under the UV light. All the plates made of D1 (J23101+I13504) GFP-colonies emitted the green light. The overnight liquid cultures of D1,1 and D1,3 showed blurry liquid but D1,2 was clear so it was discarded.

Started working with minipreparations of plasmids from the overnight cultures of D1,1 and D1,3.
The overnight cultures were transfered into 2 ml eppendorf-tubes and the cells were pelleted with the eppendorf-centrifuge (Eppendorf AG Inc. Hamburg, Centrifuge 5418, 12000 rpm, 2 mins). Added 250 ul Resuspension buffer A1 and vortexed the mixture until the cells resuspended. Added 250 ul Lysis buffer A2 (Sodium hydroxide solution 0,5-2,0 %) and inverted the tubes 5 times, which transferred liquid to blue-colored. Incubated 2 mins at room temperature for cell lysis, and added 350 ul Neutralization Buffer A3 (Guanidine hydrochloride 36-50 %) and inverted the tubes until the liquid was colorless. Buffer produced mucous sediment in the liquid, which seemed to be dead cell's remains. Centrifuged for 3 mins at 12000 RPM to pellet precipitate. Loaded clear supernatant onto the NucleioSpin Plasmid EasyPure Columns which were put into collection tubes (2 mL, Machenery-Nagel Inc., plastic information not available). Centrifuged for 30 s at the speed of 8000 rpm and discarded the flow-through. Added 450 ul Wash Buffer AQ to the spin column, and centrifuged for 1 min at the speed of 12000 rpm. Repeated the last step one more time in order to get the spin columndry. Added 50 ul Elution Buffer AE (5 mM Tris/HVl, pH 8,5) onto the middle of the column and incubated for 1 min. Centrifuged the mixture for 1 min at the speed of 12000 rpm, and the flowthrough was transparent. The purified liquid was measurede by NanoDrop:

Markins of tubes were D1A and D1B.

Nanodrop results:

Table1

Construct	Concentration (ng/ul)	A260/A280
D1A (J23101+I13504)	51,9	1,86
D1B (J23101+I13504)	136,2	1,85

6.8.2015 Interlab measurement

THURSDAY, 8/6

Made the following restriction mixtures to form D3: AHD3+AHD4+AHD5:

Table1

DNA sample	Volume of insertion (ul)
AHD3 R (J23117)	5,084477143
AHD4 R (I13504)	7,136864
AHD5 R (pSB1C3)	5,0

Added the required amounts of insertion,, 2ul of 10x T4 DNA ligase buffer and 0,2 ul T4 DNA ligase to each tube and balanced total volume to be 20 ul with nuclease-free water. After the addition of ligase, there was 20 min pause when the tubes were in ice before starting incubating 20 mins at 22C. The ligase was inactivated at 65C for 10 mins and put on ice.

Made the transformation for four ligations: D1 A and D1 B.
Added 50 ul of ice cold compenents cells into pre-chilled 2ml tube and 2 ul of resuspended DNA to the same tube, and mixed with pipette gently. Liquid was transparent and there was some moisture on the tube walls. The cells were incubated on ice for 30 mins. The tubes were put in a water bath (42C) for 60s. After the heat shock the cells were on ice incubation for 5 minutes. Incubated the cells at 37C for 1 hr with shaking (230 RPM). At this point there seemed to be growth in tubes.

7.8.2015 Interlab measurement

FRIDAY, 8/7

Checked yesterday's transformants of D3 under the UV light after 14 h plate incubation (37 C). No GFP-colonies detected, so the plates were put back in the incubator and will be taken out afternoon. No GFP colonies were found after the second incubation.